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(54) Title: OLIGONUCLEOTIDES HAVING CONJUGATES ATTACHED AT THE 2'-POSITION OF THE SUGAR MOIETY

(57) Abstract

An oligonucleotide wherein at least one nucleotide unit thereof is substituted at the 2' position with a moiety X, wherein X is $-(L)_n-R_1$. L is a linker group, and n is 0 or 1. R_1 is a moiety which improves uptake of the oligonucleotide into the cell and/or increases the stability of the oligonucleotide. The oligonucleotides may be employed for binding to an RNA, a DNA, a protein, or a peptide to inhibit or prevent gene transcription or gene expression, to inhibit or stimulate the activities of target molecules, or the oligonucleotides may be employed as diagnostic probes for determining the presence of specific DNA or RNA sequences or proteins.

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OLIGONUCLEOTIDES HAVING CONJUGATES ATTACHED AT THE 2'-POSITION OF THE SUGAR MOIETY

This invention relates to modified oligonucleotides which may bind to a DNA, an RNA, a protein, or a polypeptide, for use as a therapeutic agent. More particularly, this invention relates to oligonucleotides having conjugates attached at the 2' position of the sugar moiety of one or more nucleotide units.

Oligonucleotides may be of value as therapeutic agents for the treatment of a wide variety of diseases. Oligonucleotides offer the potential for a high degree of specificity by virtue of their capability for base pairing with complementary nucleic acid strands. Natural oligonucleotides, however, are relatively ineffective as therapeutic agents due to their poor penetrability into the cell, and their rapid degradation by enzymes inside the cell. Therefore, relatively high concentrations of natural oligonucleotides are needed in order to achieve a therapeutic effect.

The scientific literature contains some examples of modifications of the 2' or 3' position of a nucleotide sugar. For example, Furukawa et al. in Chem. Pharm. Bull., Vol. 13, pg. 1273 (1965) described the synthesis of 2'-O-methyl derivatives of nucleotides. Other investigators ahve described the modification of the 2' or 3' position of the nucleotide sugars at the 3' terminus of oligonucleotides.

Various methods for the synthesis of 2'-alkoxy oligonucleotides have been reviewed by Ohtsuka and Iwai in Synthesis and Applications of DNA and RNA; Narang, S.A., Ed.; Academic Press: Orlando, pg.115 (1987) and by Reese in Nucleic Acids and Molecular Biology; Springer - Verlag:

Berlin, Vol. 3, pg. 164 (1989). These methods involve protection of the 2'-hydroxyl function with a suitable protecting group. Recently, Sproat and his coworkers presented a synthesis of 2'O-alkyl oligoribonucleotides and Kawasaki et al. reported the preparation of 2'-deoxyribo fluorine-modified oligonucleotides at the International Conference on Nucleic Acid Therapeutics, Clearwater Beach, Florida, January 13-17, 1991. At the same conference Bhan et al. described the synthesis of 2'-O-methylribonucleotide methylphosphonate and a 3',5'-dinucleotide of 2'-amino-2'-deoxyuridine and 2'-deoxyuridine. They also reported that the presence of the 2'-O-methyl group increased resistance to spleen phosphodiesterase and also enhanced the binding to the complementary RNA strand. 2'-O-nonyl, 2'-O-aminoalkyl-, 2'-O-tert-butyldimethylsilyl-, and 2'-O-imidazolylpropyl-adenosine-modified oligonucleotides were reported by Guinosso et al. in Nucleosides and Nucleotides, Vol. 10, pgs. 259-262 (1991).

Derivatization of oligonucleotides with intercalating agents such as 2-methoxy-6-chloroacridine at the 3' terminus has been described by Toulme, et al., in Proc. Natl. Acad. Sci. U.S.A., Vol. 83, pg. 1227 (1986) using phosphotriester chemistry. They reported that this kind of modification increases the melting temperature (Tm) when hybridized to a complementary oligonucleotide, improves the biological activity and permits the use of shorter oligonucleotides than would otherwise be required. Their synthesis involves linking of 3'-phosphate of the 3'-terminal nucleotide with the 9-amino group of 2-methoxy-6-chloro-9-aminoacridine through a pentamethylene bridge. Durand et al., in Nucleic Acids Res., Vol. 17, pg. 1823 (1989) have described the 3'-conjugation of modified oligonucleotides such as methylphosphonates and methyl esters. Gautier et al. have

described in Nucleic Acids Res., Vol. 15, pg. 6625 (1987) the linkage of α- as well as β-anomeric oligonucleotides with oxazolopyridocarbazoles via the 3'-terminus using an aminoalkyl linker.

Knorre, et al. in Russ. Chem. Rev., (English Transl.), Vol. 54, pg. 836 (1985) and Vlassov, et al. in Gene, 72, 313 (1988) have reported the attachment of alkylating groups to the 3'-terminus via a 2',3'-acetal linkage with an aromatic (2-chloroethyl) amino group. Le Doan et al. have described the derivatization of oligonucleotides at the 3'-position of the sugar by DNA cleaving agents such as porphyrin in Nucleic Acids Res., Vol. 15, pg. 8643 (1987) and in Biochemistry, Vol. 25, pg. 6736 (1986). Enzyme-oligonucleotide conjugates in which the enzyme is attached to the 3'-position of the sugar at the 3'-terminus of the oligonucleotide have also been described by Schultz et al. in J. Am. Chem. Soc., Vol. 110, 1614 (1988) and in Science, Vol. 238, pg. 1401 (1987).

A solid phase synthesis of oligonucleotides with a 3'-peptide unit was described by Haralambidis, et al. in Tetrahedron Lett., Vol. 28, pg. 5199 (1987). Lemaitre, et al. has disclosed in Proc. Natl. Acad. Sci. U.S.A., Vol. 84, pgs. 648-652, (1987), and in Nucleosides and Nucleotides, Vol. 6, pg. 311 (1987) oligonucleotide-poly-L-lysine conjugates for improving the transport properties of oligonucleotides. However, this approach is not effective in all cells and polylysine is toxic at higher concentrations.

Letsinger, et al. in Proc. Natl. Acad. Sci. U.S.A., Vol. 86, pgs. 6553-6556 (1989) reported that some cholesterol conjugates of natural oligonucleotides and phosphorothioate oligonucleotides were taken up by the cell more rapidly than their unmodified counterparts. These

oligonucleotides contain the cholesterol group attached to an internucleotide bond. These conjugates were synthesized by reacting a support-bound dinucleotide phosphate derivative with reactive cholesteryl amine in the presence of an oxidizing agent such as carbon tetrachloride. Boutorin, et al. in FEBS Letters, Vol. 254, pg. 129 (1989) described the synthesis of 3'-cholesterol conjugates which involved the preparation of a 3'-phosphorylated oligonucleotide followed by reaction with cholesterol to yield the corresponding 3'-phosphodiester.

It is an object of the present invention to provide a modified oligonucleotide having improved uptake into a cell, and increased stability inside the cell, as well as facilitating the attachment of modifying conjugate groups to the oligonucleotide.

In accordance with an aspect of the present invention, there is provided an oligonucleotide wherein at least one nucleotide unit thereof is substituted at the 2' position of the sugar moiety with a moiety X, wherein X is $(L)_n-R_1$. L is a linker group and n is 0 or 1. R_1 is a moiety which improves uptake of the oligonucleotide into the cell and/or increases the stability and/or pharmacological activities of the oligonucleotide; for example, increased stability against one or more nucleases.

R_1 is selected from the group consisting of (a) amino acids, including D-amino acids and L-amino acids; (b) peptides, polypeptides, and proteins; (c) dipeptide mimics; (d) sugars; (e) sugar phosphates; (f) neurotransmitters; (g) hormones; (h) poly (hydroxypropylmethacrylamide); (i) polyethylene imine; (j) dextrans; (k) polymaleic anhydride; (l) cyclodextrins; (m) starches; (n) steroids, including sterols such as, but not limited to, cholesterol; (o) acridine; and (p) vitamins, with the proviso that the vitamin is not biotin.

The term "oligonucleotide" as used herein means that the oligonucleotide may be a ribonucleotide, deoxyribonucleotide, or a mixed ribonucleotide/deoxyribo-nucleotide; i.e., the oligonucleotide may include ribose or deoxyribose sugars or a mixture of both. Alternatively, the oligonucleotide may include other 5-carbon or 6-carbon sugars, such as, for example, arabinose, xylose, glucose, galactose, or deoxy derivatives thereof or any mixture of sugars.

The phosphorus containing moieties of the oligonucleotides of the present invention may be modified or unmodified. The phosphorus containing moiety may be, for example, a phosphate, phosphonate, alkylphosphonate, aminoalkyl phosphonate, thiophosphonate, phosphoramidate, phosphordiamidate, phosphorothioate, phosphorothionate, phosphorothiolate, phosphoramidothiolate, and phosphorimidate. It is to be understood, however, that the scope of the present invention is not to be limited to any specific phosphorus moiety or moieties. Also, the phosphorus moiety may be modified with a cationic, anionic, or zwitterionic moiety. The oligonucleotides may also contain backbone linkages which do not contain phosphorus, such as carbonates, carboxymethyl esters, acetamidates, carbamates, acetals, and the like.

The oligonucleotides also include any natural or unnatural, substituted or unsubstituted, purine or pyrimidine base. Such purine and pyrimidine bases include, but are not limited to, natural purines and pyrimidines such as adenine, cytosine, thymine, guanine, uracil, or other purines and pyrimidines, such as isocytosine, 6-methyluracil, 4,6-di-hydroxypyrimidine, hypoxanthine, xanthine, 2,6-diaminopurine, 5-azacytosine, 5-methyl cystosine, and the like.

In general, the oligonucleotide includes at least two, preferably at least 5, and most preferably from 5 to 30 nucleotide units.

In one embodiment, the at least one nucleotide unit which is substituted at the 2' position is the 3' terminal nucleotide unit.

In another embodiment, the at least one nucleotide unit which is substituted at the 2' position consists of one or more adjacent nucleotide units at the 3' end and/or the 5' end of the oligonucleotide. In yet another embodiment, the at least one adjacent nucleotide unit which is substituted at the 2' position as hereinabove described may be nucleotide units which alternate with unsubstituted nucleotide units. In another embodiment, all of the nucleotide units are substituted at the 2' position.

Amino acids which may be attached to the 2' position of the sugar moiety include, but are not limited to, alanine, methionine, leucine, isoleucine, and lysine, as well as dipeptides, polypeptides, and polymers and copolymers of the above-mentioned amino acids, such as, for example, polylysine, as well as proteins. The amino acids, or polymers thereof, may be attached to the at least one nucleotide unit by the acid functionality, the amino group, or by the side chain.

Dipeptide mimics which may be employed include, but are not limited to, aminoethyl glycine, and the cephalosporins. Cephalosporins which may be employed include, but are not limited to, cephalexin, cephradine, cefaclor, cefadroxil, cefazolin, and cefotiam.

Sugars which may be conjugated to at least one nucleotide unit of the oligonucleotide at the 2' position include, but are not limited to, 5-carbon and 6-carbon sugars. 5-carbon sugars which may be employed include

ribose, arabinose, xylose, and lyxose. 6- carbon sugars include, but are not limited to, glucose, galactose, mannose, allose, gulose, idose, talose, and altrose. Preferred sugars are glucose, galactose, and mannose.

Sugar phosphates which may be employed include, but are not limited to, mannose-6-phosphate, glucose-6-phosphate, galactose-6-phosphate, mannose-1-phosphate, glucose-1-phosphate, and galactose-1-phosphate.

Neurotransmitter conjugates at the 2' position of at least one nucleotide unit of the oligonucleotide include, but are not limited to, acetylcholine, dopamine, epinephrine, norepinephrine, and serotonin. A preferred neurotransmitter is dopamine.

Hormones which may be attached to the 2' position of the sugar moiety of at least one nucleotide unit of the oligonucleotide include, but are not limited to, peptide hormones and steroid hormones. Peptide hormones which may be employed include, but are not limited to, growth factors such as epidermal growth factor (EGF), which is a polypeptide containing 53 amino acids and is described in Savage, et al., J. Biol. Chem., Vol. 247, pg. 7609 (1972); thyrotropin releasing factor (TRH); oxytocin; and cholecystokinin. Steroid hormones which may be employed include, but are not limited to, female sex hormones such as estrogens (eg., estrone), male sex hormones such as testosterone and androsterone, and corticoids such as cortisone.

Polymers which may be attached to the 2' position of the sugar moiety of at least one nucleotide unit of the oligonucleotide include, but are not limited to, polyethyleneimine, dextran, starch, and lipophilic polymers such as poly(hydroxypropylmethacrylamide); polymaleic anhydride, cyclodextrins, and starch.

Steroids which may be attached to the 2' position of the sugar moiety of the oliogonucleotide include, but are

not limited to, the steroid hormones hereinabove described, and sterols, which include, but are not limited to, cholesterol.

Any vitamin may be attached to the 2' position of the sugar moiety of at least one nucleotide unit of the oligonucleotide, with the proviso that the vitamin is not biotin (Vitamin H). Representative examples of vitamins which may be attached to the 2' position of the sugar moiety include, but are not limited to, Vitamin A, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Vitamin C, Vitamin D, Vitamin E, folic acid, and nicotinic acid.

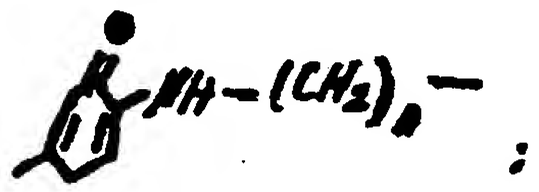
A variety of methods may be used to attach conjugate groups directly to the 2'-position of oligonucleotides. The hydroxyl group of a ribonucleotide unit in the oligomer can be used as an attachment site, and reaction with an activated ester of the conjugate group would produce an ester linkage between the oligonucleotide and the conjugate group. An amino group can be introduced into the oligonucleotide (hereinafter described as an "oligonucleotide amino group") by methods such as those outlined hereinbelow, and reaction of this amino group with an activated ester attached to the conjugate moiety would produce a conjugate linked via an amide functionality. Alternatively, a thiol group can be introduced into the oligonucleotide (hereinafter described as an "oligonucleotide thiol group") and this can be used as the attachment site. An active ester attached to the conjugate would produce a thioester, or alternatively reaction with another thiol would produce a conjugate linked by a disulfide bridge. Other substituent groups could be attached to the 2'-position by a variety of chemistries.

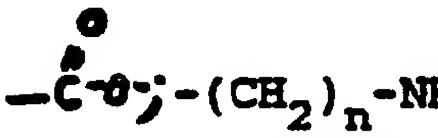
Linker arms can be employed to attach conjugate groups to oligonucleotides, and examples of such linker groups are described herein. The length of the linker arm can be varied so as to maximize the binding of the conjugate group

to the receptor, which may be enhanced when the conjugate group is well separated from the oligonucleotide.

Thus, in one embodiment, the moiety X includes a linker group L which links the moiety R_1 with the oligonucleotide. A wide variety of linker groups may be employed, depending upon the nature of the nucleotide unit, the moiety R_1, and whether the linker group is present during the synthesis of the oligonucleotide. The linker group may be a single atom, or a functional group. Examples of linkers include, but are not limited to -NH-, or amino groups, sulfur atoms, and polyvalent functional groups.

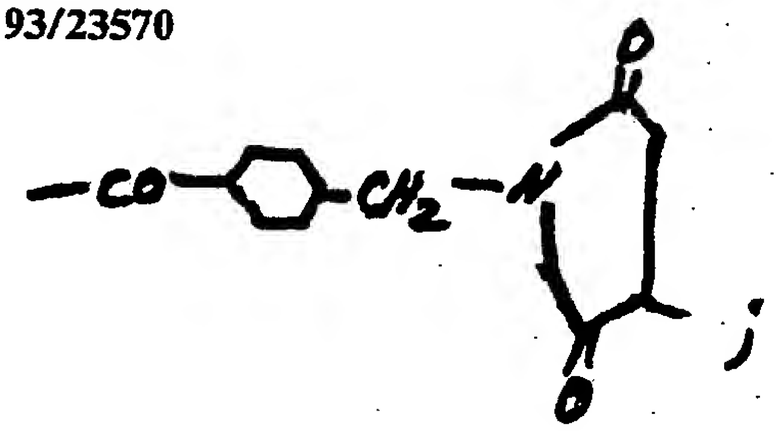
In another embodiment, the linking group is derived from a polyvalent functional group having at least one atom, and not more than about 60 atoms other than hydrogen, preferably not more than about 30 atoms other than hydrogen. The linker group in general has up to about 30 carbon atoms, preferably not more than about 20 carbon atoms, and up to about 10 heteroatoms, preferably up to about 6 heteroatoms, and in particular such heteroatoms may be oxygen, sulfur, nitrogen, or phosphorus. Representative examples of linker groups include, but are not limited to

$-CO-(CH_2)_n-NH-$; $-CO-(CH_2)_n-CO-$;  ;

 ; $-(CH_2)_n-NH-$;

$-CO-$; $-CO-CH_2-CH_2-S-S-$;; $-CH_2CH_2NHCO(CH_2)_n$ $CONH-$;

$-CH_2CH_2-NH-Q-(CH_2)_nNH-$, wherein Q is 2,5-quinondiyl;



In the above structures, n is from 1 to 20, preferably from 2 to 12, and more preferably 6.

Most preferably, however, the linker group is an -NH- group. The -NH- group may serve as a linker alone, or may be bonded to other linker groups such as those hereinabove described to form a linker group for linking the conjugate group to the oligonucleotide.

It is to be understood, however, that the means of attachment of a conjugate group to the oligonucleotide at the 2' position of the sugar moiety varies with respect to the conjugate group employed. For example, amino acids may be attached to the 2' position of an oligonucleotide via the amino group, the carboxyl group, or the side chain.

Attachment to the amino group of the amino acid can be accomplished by using a crosslinking agent such as disuccinimidyl suberate, which forms amide bonds with both the amino acid and with a 2'-oligonucleotide amino group. Other crosslinking agents with shorter or longer linker arms can also be employed in place of disuccinimidyl suberate. Attachment via the carboxyl group can be accomplished by activation using an N-hydroxysuccinimide ester or another active ester such as a p-nitrophenyl or pentafluorophenyl ester. Such esters can be reacted directly with a 2'-oligonucleotide amino group or reacted with an amino group attached to the 2'-position via a linker arm. Alternatively such active esters can be reacted with the 2'-hydroxyl group or a 2'-oligonucleotide thiol group to give conjugates linked via ester or thioester linkages,

respectively. Attachment to the side chain of an amino acid depends upon the nature of the amino acid because the side chains differ widely. Attachment to acidic or basic side chains can be carried out by methods similar to those described for the carboxyl and amino groups, whereas attachment to hydrocarbon side chains necessitates introduction of new attachment sites.

Because peptides and proteins possess the same functional groups as amino acids, the same chemistries as described above with respect to amino acids can be used for attachment of peptides. Attachment at the C-terminal residue can be achieved by coupling to an amino group on the 2'-position of the oligonucleotide, using a coupling agent such as carbodiimide. Attachment to the N-terminal amino acid can be carried out using a crosslinking agent such as disuccinimidyl suberate which will form amide bridges with the 2'-amino group of the oligonucleotide and also with the amino group on the peptide. Peptide hormones, such as those hereinabove described often possess amino acids with basic sidechains such as lysine which are also logical sites for attachment to the oligonucleotide using the same chemistry as outlined above.

Dipeptide mimics such as aminoethyl glycine possess amino and carboxyl groups, both of which can be used for attachment. Attachment via the amino group can be accomplished by using a crosslinking agent such as disuccinimidyl suberate, which forms amide bonds with both the dipeptide mimic and with a 2'-oligonucleotide amino group. Other crosslinking agents can also be employed in place of disuccinimidyl suberate. Attachment via the carboxyl group can be accomplished by activation using an active ester as is hereinabove descibed with respect to amino acids. Such esters could be reacted directly with a

2'-amino group on the oligonucleotide, or reacted with an amino group attached to the 2'-position via a linker arm. Alternatively, such active esters can be reacted with the 2'-hydroxyl group or a 2'-thiol group to give conjugates linked via ester or thioester linkages, respectively.

Aminocephalosporins have been shown to be transported into cells via the dipeptide transport system of intestinal brush border membranes as was described in the Journal of Biological Chemistry, Volume 261, pgs. 14130-14134 (1986), and thus can be considered as dipeptide mimics. These can be attached to oligonucleotides to produce conjugates which can be taken up by dipeptide transport systems and thus be internalized more efficiently. These molecules possess amino and carboxyl groups, both of which can be used as attachment sites for conjugation using methods outlined above. Attachment to the amino group of the cephalosporin can be accomplished by using a crosslinking agent which forms amide bonds with both the cephalosporin and with the 2'-oligonucleotide amino group. Attachment via the carboxyl group can be accomplished by activation using an active ester which can be reacted directly with a 2'-oligonucleotide amino group, or reacted with an amino group attached to the 2'-position via a linker arm. Alternatively, such active esters can be reacted with the 2'-hydroxyl group or the 2'-oligonucleotide thiol group to give conjugates linked via ester or thioester linkages, respectively. In addition, these compounds have other ring substituent groups which can be used as linkage sites without interfering with the amino and carboxyl functionalities.

Sugars have several hydroxyl groups which can be used for attachment to oligonucleotides. In one embodiment, one may react a partially protected sugar derivative such as

1,2,3,4-tetracetyl-D-glucopyranose with a 2'-phosphorylated oligonucleotide using a condensing agent such as dicyclohexylcarbodiimide or tri-isopropylbenzenesulfonyl chloride. Another approach is to use a crosslinking agent to attach a linker arm bearing an active ester group to a 2'-oligonucleotide amino group. This active ester would be capable of reacting with a hydroxyl group of the sugar to give an ester linkage. Yet another approach is to couple an active ester derivative of a sugar acid to a 2'-terminal oligonucleotide amino group to produce an amide linkage. Still another approach is to react a sugar isothiocyanate such as 1,2,3,4-tetracetyl- D-glucopyranose-1-isothiocyanate with a 2'-oligonucleotide amino group .

Sugar phosphates may be attached to oligonucleotides by condensation reactions via the phosphate group. For example, reaction of 1,2,3,4-tetracetyl-glucose-6-phosphate with the 2'-terminal amino group of an oligonucleotide using a condensing agent such as tri-isopropylbenzensulfonyl tetrazolide will produce a conjugate in which the sugar phosphate is linked to the oligonucleotide via a phosphoramidate linkage. Alternatively 1,2,3,4-tetracetyl-glucose-6-phosphate can be coupled to the 2'-hydroxyl group of a ribonucleotide using the same condensing agent to produce a conjugate linked via a phosphodiester.

A conjugate with acetylcholine can be prepared by reaction of choline with bromoacetic anhydride to give bromoacetylcholine, which could be linked to a 2'-oligonucleotide thiol group. The thiol can be attached directly to the sugar or to a linker arm attached to the 2'-position.

Dopamine, norepinephrine and serotonin all have phenolic groups and amino groups which can be used as

attachment sites. Attachment via the amino group could be accomplished using a crosslinking agent such as disuccinimidyl suberate, providing that the oligonucleotide contains a 2'-oligonucleotide amino group. Various other crosslinking agents may also be employed and the length of linker could be adjusted for optimal activity. Other crosslinking agents may be used for coupling of a 2'-oligonucleotide thiol group to the amino group of dopamine, norepinephrine or serotonin. The phenolic groups of dopamine, norepinephrine or serotonin may also be used as attachment sites. A linker arm with an active ester can be attached to the 2'-position of the oligonucleotide as previously described, and reaction with an N-protected derivative of dopamine, norepinephrine or serotonin would produce a phenolic ester. Subsequent removal of the N-protecting group would produce the neurotransmitter conjugate.

Steroids, which include steroid hormones and sterols such as cholesterol, may be attached to the 2'-position of an oligonucleotide by a variety of chemistries depending upon the available substituent groups on the steroid or sterol. Estrone may be activated by formation of a chloroformate and then reacted at the amino group at the 2'-position with a protected or unprotected oligonucleotide. Alternatively, estrone can be reacted with epsilon bromohexanoic acid to give an acid which can then be converted into an active ester and reacted with a 2'-oligonucleotide amino group. Similar procedures can be employed for the coupling of male sex hormones such as testosterone or androsterone, and corticoids such as cortisone.

Attachment of poly (hydroxypropylmethacrylamide) may be accomplished by synthesis of a monomer with a linker arm

such as N-methacryloyl-epsilon-amino caproic acid-p-nitrophenyl ester as described by Kopecek and Rejmanova in the Journal of Polymer Science, Vol. 66, pgs. 15-32 (1979), and copolymerizing a mixture of this material with N-(2-hydroxypropyl)methacrylamide (HPMA). This will produce a poly-HPMA derivative with linker arms bearing reactive p-nitrophenyl ester groups, which can be reacted with 2'-oligonucleotide amino groupto give an oligonucleotide-poly-HPMA conjugate. Poly-HPMA derivatives with other side chains may also be used in this manner, and drugs such as adriamycin have been attached to poly-HPMA as described by Kopececk and Duncan in the Journal of Controlled Release, Vol. 6, pgs. 315-327 (1987).

Polyethyleneimine may be attached to an oligonucleotide at the 2'-position by reaction of the oligonucleotide with a crosslinking agent such as disuccinimidyl suberate, followed by reaction of the product with polyethyleneimine. Other similar crosslinking agents with shorter or longer linker arms can also be employed in place of disuccinimidyl suberate if it is deemed necessary to vary the length of the linker arm.

Dextran may be attached to an oligonucleotide by reaction with 6-bromohexanoic acid as described by Pietta, et al. in Preparative Biochemistry, Vol. 14, pgs. 313-329 (1984) to give a carboxylic acid which can be converted into an active ester derivative. The active ester can then be reacted with a 2'oligonucleotide amino to give a 2'-dextran conjugate. Alternatively, dextran can be partially oxidized with periodate to give a dialdehyde which can be reacted with the 2'-oligonucleotide amino group to give a Schiff base which can then be reduced. Other methods such as activation with cyanogen bromide or a chloroformate ester can be used and have been reviewed by Schacht in Industrial

Polysaccharides: Genetic Engineering, Structure/Property Relations and Applications, M. Yalpani ed., (1987) pgs. 389-400 and by Yalpani and Brooks in Journal of Polymer Science, Vol. 23, pgs. 1395-1405 (1985). Several of the above methods may also be applied to the formation of conjugates with other carbohydrate molecules such as cyclodextrin and starch.

Acridine may be attached to the oligonucleotide at the 2'-position of the sugar moiety by reacting 6,9-dichloro-2-methoxyacridine with 6-aminocaproic acid to produce a 9-substituted acridine-caproic acid adduct, which may be condensed with a 2'-oligonucleotide amino group using a water soluble carbodiimide as the coupling agent. Alternatively, the acridine-aminocaproic acid adduct can be converted into an active ester, and then coupled to the 2'-oligonucleotide amino group. In another alternative, 6,9-dichloro-2-methoxyacridine is reacted with a diamine such as 1,6-hexanediamine to produce an acridine with an amino-containing linker arm at the 9-position. This compound can then be coupled to the 2'-position of an oligonucleotide using a crosslinking agent such as disuccinimidyl suberate. The length of the linker arm can be varied depending upon the particular application.

The method for attachment of vitamins depends upon the nature of the vitamin and its available functional groups. Several vitamins including Vitamin A, thiamine (Vitamin B_1), riboflavin (Vitamin B_2), pyridoxine (Vitamin B_6), Vitamin B_{12}, ascorbic acid (Vitamin C), and Vitamin D possess hydroxyl groups which can be used as linkage sites for attachment of a linker arm. Another group of vitamins including folic acid and nicotinic acid, have carboxyl groups which can be used as attachment sites by coupling to a 2'oligonucleotide -amino group, or to an amino group

attached to a linker arm at the 2'-position, using a carbodiimide coupling agent. All of the above can be modified at a position in the carbon skeleton to produce analogs which can be attached to the 2'-position of oligonucleotides.

The oligonucleotides of the present invention may be employed to bind to RNA sequences by Watson-Crick hybridization, and thereby block RNA processing or translation. For example, the oligonucleotides of the present invention may be employed as "antisense" complements to target sequences of mRNA in order to effect translation arrest and selectively regulate protein production.

The oligonucleotides of the present invention may be employed to bind double-stranded DNA to form triplexes, or triple helices. Such triplexes inhibit the replication or transcription of DNA, thereby disrupting gene replication or transcription. Such triplexes may also protect DNA binding sites from the action of enzymes such as DNA methylases.

The RNA or DNA target of interest, to which the oligonucleotide binds, may be present in a prokaryotic or eukaryotic cell, a virus, a normal cell, or a neoplastic cell. The sequences may be bacterial sequences, plasmid sequences, viral sequences, chromosomal sequences, mitochondrial sequences, or plastid sequences. The sequences may include open reading frames for coding proteins, mRNA, ribosomal RNA, snRNA, hnRNA, introns, or untranslated 5'- and 3'-sequences flanking open reading frames. The target sequence may therefore be involved in inhibiting production of a particular protein, enhancing the expression of a particular gene by inhibiting the expression of a repressor, or the sequences may be involved in reducing the proliferation of viruses or neoplastic cells.

The oligonucleotides may be used in vitro or in vivo for modifying the phenotype of cells, or for limiting the proliferation of pathogens such as viruses, bacteria, protists, Mycoplasma species, Chlamydia or the like, or for killing or interfering with the growth of neoplastic cells or specific classes of normal cells. Thus, the oligonucleotides may be administered to a host subject in a diseased state to inhibit the transcription and/or expression of the native genes of a target cell. Therefore, the oligonucleotides may be used for protection from, or treatment of, a variety of pathogens in a host, such as, for example, enterotoxigenic bacteria, Pneumococci, Neisseria organisms, Giardia organisms, Entamoebas, neoplastic cells, such as carcinoma cells, sarcoma cells, and lymphoma cells; specific B-cells; specific T-cells, such as helper cells, suppressor cells, cytotoxic T-lymphocytes (CTL), natural killer (NK) cells, etc.

The oligonucleotides may be selected so as to be capable of interfering with transcription product maturation or production of proteins by any of the mechanisms involved with the binding of the subject composition to its target sequence. These mechansims may include interference with processing, inhibition of transport across the nuclear membrane, cleavage by endonucleases, or the like.

The oligonucleotides may be complementary to such sequences as sequences expressing growth factors, lymphokines, immunoglobulins, T-cell receptor sites, MHC antigens, DNA or RNA polymerases, antibiotic resistance, multiple drug resistance (mdr), genes involved with metabolic processes, in the formation of amino acids, nucleic acids, or the like, DHFR, etc. as well as introns or flanking sequences associated with the open reading frames.

The following table is illustrative of some additional applications of the subject compositions.

Area of Application	Specific Application Targets
Infectious Diseases:	
Antivirals, Human	HIV, HSV, CMV, HPV, VZV infections
Antivirals, Animal	Chicken Infectious Bronchitis Pig Transmissible Gastroenteritis Virus infections
Antibacterial, Human	Drug Resistance Plasmids
Antiparasitic Agents	Malaria Sleeping Sickness (Trypanosomes)
Cancer	
Direct Anti-Tumor Agents	Oncogenes and their products
Adjunctive Therapy	Drug Resistance genes and their products
Auto Immune Diseases	
T-cell receptors	Rheumatoid Arthritis Type I Diabetes Systemic Lupus Multiple sclerosis
Organ Transplants	OKT3 cells causing GVHD

The oligonucleotides of the present invention may be employed for binding to target molecules, such as, for example, proteins including, but not limited to, ligands, receptors, and or enzymes, whereby such oligonucleotides inhibit the activity of the target molecules.

The oligonucleotides of the present invention are administered in an effective binding amount to an RNA, a DNA, a protein, or a peptide. Preferably, the oligonucleotides are administered to a host, such as a human or non-human animal host, so as to obtain a concentration of oligonucleotide in the blood of from about 0.1 to about 100 μmole/l. It is also contemplated that the oligonucleotides may be administered in vitro or ex vivo as well as in vivo.

The oligonucleotides may be administered in conjunction with an acceptable pharmaceutical carrier as a pharmaceutical composition. Such pharmaceutical compositions may contain suitable excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Such oligonucleotides may be administered by intramuscular, intraperitoneal, intraveneous, or subdermal injection in a suitable solution. Preferably, the preparations, particularly those which can be administered orally and which can be used for the preferred type of administration, such as tablets, dragees and capsules, and preparations which can be administered rectally, such as suppositories, as well as suitable solutions for administration parenterally or orally, and compositions which can be administered buccally or sublingually, including inclusion compounds, contain from about 0.1 to 99 percent by weight of active ingredients, together with the excipient. It is also contemplated that the oligonucleotides may be administered topically.

The pharmaceutical preparations of the present invention are manufactured in a manner which is itself well known in the art. For example, the pharmaceutical preparations may be made by means of conventional mixing, granulating, dragee-making, dissolving or lyophilizing processes. The process to be used will depend ultimately on the physical properties of the active ingredient used.

Suitable excipients are, in particular, fillers such as sugar, for example, lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example, tricalcium phosphate or calcium hydrogen phosphate, as well as binders such as starch or paste, using, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carboxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinyl pyrrolidone. If desired, distintegrating agents may be added, such as the above-mentinoed starches as well as carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are flow-regulating agents and lubricants, such as, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, and/or polyethylene glycol. Dragee cores may be provided with suitable coatings which, if desired, may be resistant to gastric juices. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetylcellulose phthalate or hydroxypropylmethylcellulose phthalate, are used. Dyestuffs

and pigments may be added to the tablets of dragee coatings, for example, for identification or in order to characterize different combinations of active compound doses.

Other pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the oligonucleotide in the form of granules which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Possible pharmaceutical preparations which can be used rectally include, for example, suppositories, which consist of a combination of the active compounds with a suppository base. Suitable suppository bases are, for example, natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols, or higher alkanols. In addition, it is also posible to use gelatin rectal capsules which consist of a combination of the active compounds with a base. Possible base materials include, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble or water-dispersible form. In addition, suspensions of the active compounds as appropriate oil injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, sesame oil, or synthetic fatty acid esters, for

example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethyl cellulose, sorbitol and/or dextran. Optionally, the suspension may also contain stabilizers.

Additionally, the compounds of the present invention may also be administered encapsulated in liposomes, wherein the active ingredient is contained either dispersed or variously present in corpuscles consisting of aqueous concentric layers adherent to lipidic layers. The active ingredient, depending upon its solubility, may be present both in the aqueous layer, in the lipidic layer, or in what is generally termed a liposomic suspension. The hydrophobic layer, generally but not exclusively, comprises phospholipids such as lecithin and sphingomycelin, steroids such as cholesterol, surfactants such as dicetylphosphate, stearylamine, or phosphatidic acid, and/or other materials of a hydrophobic nature. The diameters of the liposomes generally range from about 15 nm to about 5 microns.

The oligonucleotide conjugates of the present invention may also be employed as diagnostic probes. In this approach the conjugate group serves as a nonradioactive reporter group for the detection of nucleic acid sequences of interest. In one embodiment, the DNA-containing sample to be analyzed is immobilized on an inert solid support such as a nitrocellulose membrane and then annealed with the oligonucleotide conjugate. This annealing procedure allows the oligonucleotide to bind to the DNA provided that the base sequences are complementary to each other. After a series of washing steps the duplex of the oligonucleotide conjugate with the DNA is exposed to a complex of an antibody to the conjugate group attached to an enzyme such as alkaline phosphatase. After a further series of washing

steps, the DNA-antibody-enzyme complex is detected by exposure to a chromogenic substrate which generates a purple-blue color in the area of the bound complex.

Several other conjugate groups have been used in this manner. For example, oligonucleotide diagnostic probes have been prepared by attachment of digoxigenin to the 5-position of pyrimidine bases as reported by Muhlegger et al. in Nucleosides and Nucleotides, Vol. 8, pages 1161-1163 (1989), and conjugates of the 2,4-dinitrophenyl group have been reported by Vincent et al. in Nucleic Acids Research, Volume 10, pages 6787-6796 (1982).

The oligonucleotide conjugates can also be used as diagnostic probes to interact with RNA's in a sample provided that the target RNA has a sequence complementary to the oligonucleotide conjugate sequence. If both DNA and RNA are present in the sample and it is desired to measure only DNA, the sample can be treated with RNase prior to addition of the oligonucleotide conjugate. If it is desired to measure only RNA, the sample can be treated with DNase prior to addition of the oligonucleotide conjugate. The oligonucleotide conjugates can also be used as diagnostic probes to interact with proteins in a sample provided that the target protein binds tightly or specifically to the conjugated oligonucleotide because of the sequence of the conjugated oligonucleotide. For example, the glucocorticoid receptor protein has been demonstrated to bind with high affinity to the sequence $GGTACAN_3TGTTCT$, wherein N is any purine or pyrimidine base. (R.M. Evans, Science, Vol. 240, pgs. 889 (1988)). A double stranded oligonucleotide in which one strand is an oligonucleotide conjugate of the present invention could be used as a diagnostic probe to measure glucocorticoid receptor protein in a sample. Other DNA-binding proteins can be similarly measured. Bock et al.

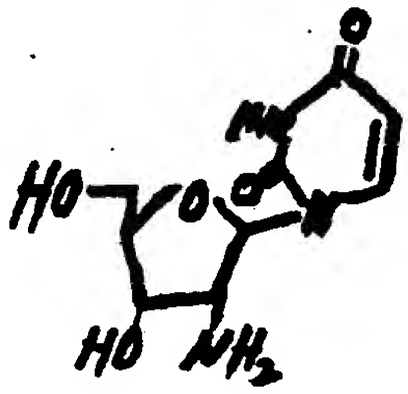
(Nature, Vol. 355, pages 564-566, (1992) have demonstrated that the protein thrombin binds tightly to DNA oligonucleotides containing the concensus sequence $GGTTGG(N_3)GGTTGG$. An oligonucleotide of the present invention containing ribonucleotides or deoxyribonucleotides of a given sequence might thus be used to detect a protein which binds that sequence.

The invention will now be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Synthesis of 2'-trifluoroacetamido-2' deoxyuridine

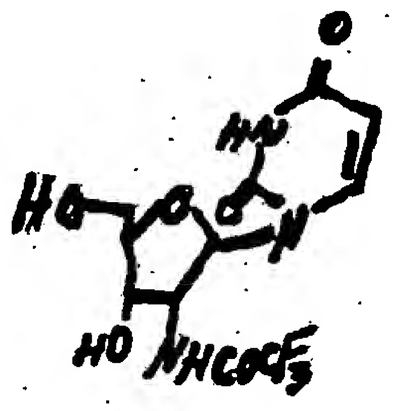
S-ethyl trifluorothioacetate (0.359 ml; 2.8 mmol) was added dropwise at ambient temperature to a stirred solution of:



(1)

[prepared from 2,2'-anhydro-1-(β-D-arabinofuranosyl) uracil by the procedure of Verheyden, et al., J. Org. Chem., Vol. 36, pg. 250 (1971)] in methanol. The reaction flask was loosely stoppered and the contents stirred for 30 hours. Another portion of S-ethyl trifluorothioacetate (0.05 ml; 0.39 mmol) was added and stirring was continued for an additional 23 hours. The mixture was concentrated to dryness in vacuo, and the residue was dissolved in ethyl

acetate and purified by silica gel flash column chromatography using ethyl acetate as eluant to give 2'-trifluoroacetamido-2'-deoxyuridine, having the following structure:



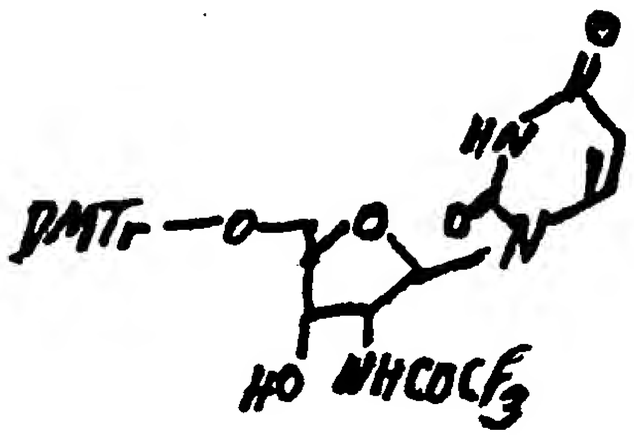
(2)

as a white fluffy solid. An aliquot of 2'-trifluoroacetamido-2'-deoxyuridine was dissolved in a solution of 5% ninhydrin in 95% ethanol and then heated to give a negative ninhydrin test.

Example 2

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2'-trifluoroacetamido-2'-deoxyuridine

4,4'-dimethoxytrityl (DMTr) chloride (95% pure; 0.478g; 1.34 mmol) was added to a stirred solution of 2'-trifluoroacetamido-2'-deoxyuridine in dry pyridine (5 ml) at 0°C. The reaction flask was stoppered and stirring continued at 0°C for 0.5 hour and then at ambient temperature for 4.5 hours. The flask was then cooled in ice and the mixture was quenched with methanol (1 ml). The mixture was stirred at 0°C for 5 minutes and then at ambient temperature for 20 minutes, followed by concentration in vacuo. The residue was coevaporated with toluene (2 x 15 ml) in vacuo and purified by silica gel flash column chromatography using ethyl acetate/pentane (1:1) as eluant to give a light yellow solid product of 5'-O-(4,4'-dimethoxytrityl)-2'-trifluoroacetamido-2'-deoxyuridine having the following structural formula:



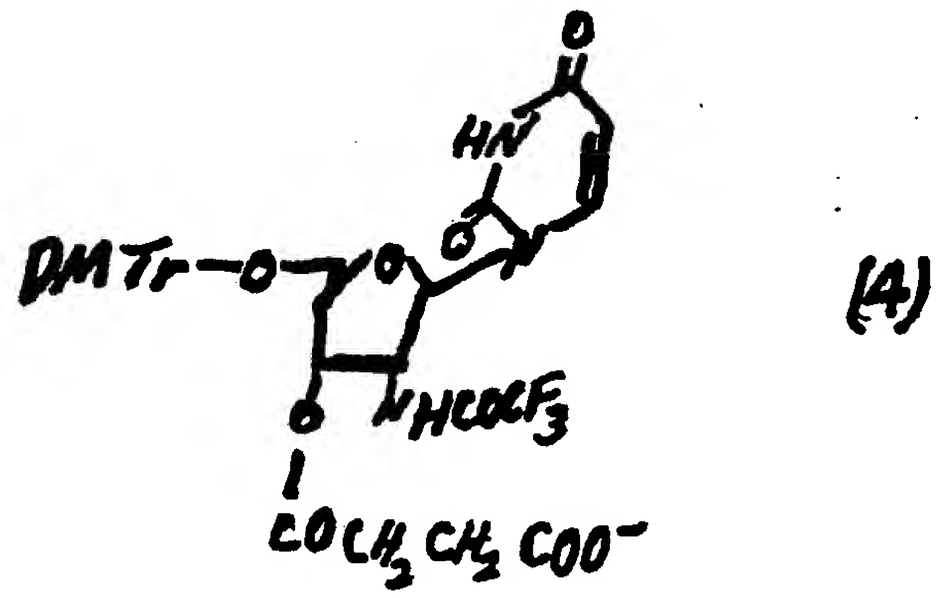
(3)

An aliquot of 5'-O-(4,4'-dimethoxytrityl)-2'-trifluoroacetamido-2'-deoxyuridine gave a negative ninhydrin test.

Example 3

Synthesis of 5'-O-(4,4'-dimethoxytrityl)-2'-trifluoroacetamido-2'-deoxyuridine-3'-O-monosuccinate and conjugation thereof to long chain aminoalkyl controlled pore glass

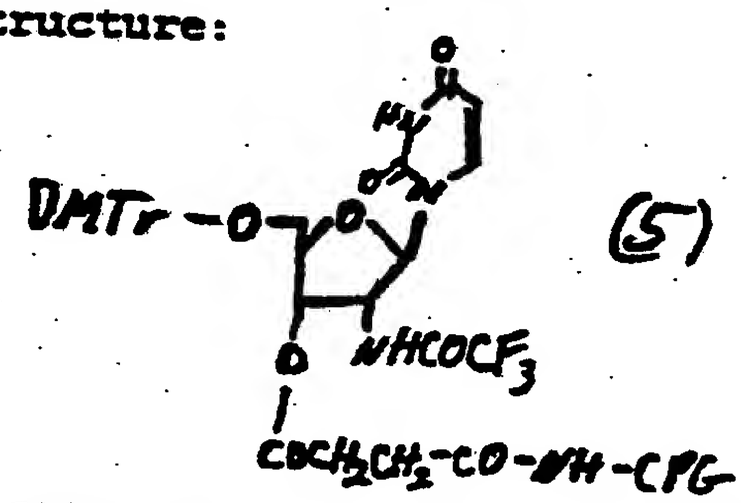
4-dimethylaminopyridine (0.025 g; 0.2 mmol) and succinic anhydride (0.020g; 0.2 mmol) were added successively to a solution of 5'-O-(4,4'-dimethoxytrityl)-2'-trifluoroacetamido-2'-deoxyuridine (0.128g; 0.2 mmol) in dry pyridine (5 ml) at 25°C. The reaction mixture was stirred for 1 day and then concentrated in vacuo to give the 3'-O-monosuccinate derivative having the following structural formula:



(4)

as a viscous oil. This oil was dissolved in dry dichloromethane (1.6 ml), mixed with dicyclohexyl carbodiimide (0.124 g; 0.6 mmol) and then stirred for 5 minutes at ambient temperature. Long chain aminoalkyl controlled pore glass (LCAA-CPG; 0.4g; loading of functional group equals 89.5 µmole/g) was added and the mixture was shaken on an orbital shaker (3500 rpm) for 17 hours.

The mixture was diluted with dry dichloromethane (3ml), filtered and the solid was thoroughly washed with dry tetrahydrofuran (THF) followed by dry ether and dried over P_2O_5 in vacuo to yield 0.405g of solid. Capping of unreacted functional groups was carried out by successively adding acetic anhydride (0.2 ml) and 4-dimethyl amino pyridine (DMAP) (4 mg) to a suspension of derivatized LCAA-CPG in pyridine (1.2 ml) and then the mixture was shaken occasionally at ambient temperature for 1.75 hours. The mixture was filtered to give a supported nucleotide having the following structure:



(5)

The mixture was filtered and the derivatized solid support (5) was thoroughly washed with dry tetrahydrofuran (THF) followed by anhydrous ether and dried over P_2O_5 in vacuo to give 0.4g of white solid. Loading of nucleoside in LCAA-CPG was 41.9 µmol/g and was determined by dimethoxytrityl cation assay as described by Atkinson and Smith in Oligonucleotide Synthesis: A Practical Approach, M.J. Gait ed.; I.R.L. Press, Oxford, 1984, pg. 48 except that 3% dichloroacetic acid in dichloromethane was used

instead of perchloric acid/methanol. An aliquot of (5) gave a negative ninhydrin test.

Example 4

Synthesis of a 2' Amino Oligomer

The supported nucleotide (5) (10 mg; 0.419 µmole) was loaded into a 1 µmole size column, installed on an Applied Biosystems DNA synthesizer (Model #394), and synthesis of a modified oligonucleotide having the following structure:

AGT GTT CAG TTC CGU-2'-NH_2 (6)

was performed by using standard cyanoethyl phosphoramidite chemistry.

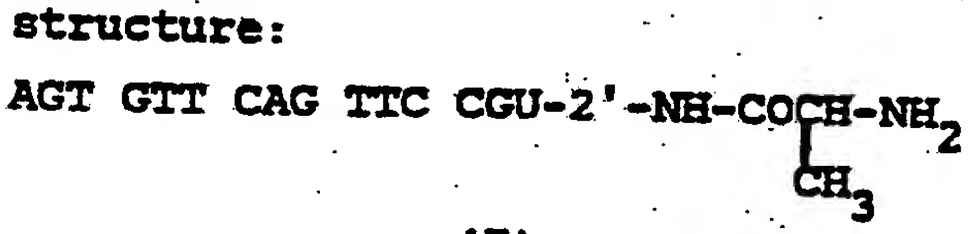
After cleavage from the solid support, the ammonia solution from the synthesizer was heated at 55°C overnight to remove the base protecting groups, and then evaporated to dryness. The residue was dissolved in 300 µl of water, and passed through a Sephadex G25 column (1 x 10 cm) which was eluted with water. The fractions containing the oligonucleotide (as determined by UV spectroscopy) were combined, and evaporated to dryness to give the crude oligonucleotide (6).

Example 5

Attachment of Alanine to a 2'-amino-oligonucleotide

A solution of the oligonucleotide (6) (75 OD_{260} units) in 0.2M sodium phosphate (pH 8.25, 195 µl) was added to an Eppendorf tube containing a solution of N-fluorenylmethoxycarbonyl-alanine succinimidate (3 mg) in dry dimethylformamide (90 µl) and the resulting mixture was stirred for 15 seconds. This solution was incubated at ambient temperature for 1 hour and then transferred into another Eppendorf tube containing a solution of

N-fluorenylmethoxycarbonyl-alanine succinimidate (2.2 mg) in dry dimethylformamide (30 µl). The mixture was again stirred for 15 seconds and incubated for 6 hours. The mixture was filtered and the filtrate purified by preparative reversed phase C-18 HPLC using a linear gradient of 0.1M triethylammonium acetate (pH 7.1)/acetonitrile as eluant. The fraction containing the desired product was collected and concentrated in vacuo to give the N-fluorenylmethoxycarbonyl protected alanine-oligonucleotide conjugate. This material was then dissolved in cold 28% ammonium hydroxide (1 ml) and incubated at ambient temperature for 5.5 hours. Nitrogen was bubbled through the mixture for 30 minutes, and the solution was then evaporated to dryness. The solid was dissolved in 1 ml of deionized water and then extracted four times with 1 ml of ethyl acetate. Traces of ethyl acetate present in the aqueous layer were removed under a stream of nitrogen, and the solution was then concentrated in vacuo to dryness. The residue was dissolved in 200 µl of deionized water, filtered, and the filtrate is subjected to preparative reversed phase C-18 HPLC using a linear gradient of 0.1M triethylammonium acetate/acetonitrile as eluant, and the desired fraction containing the alanine-oligonucleotide conjugate was collected and concentrated in vacuo to give an alanine-oligonucleotide conjugate having the following structure:



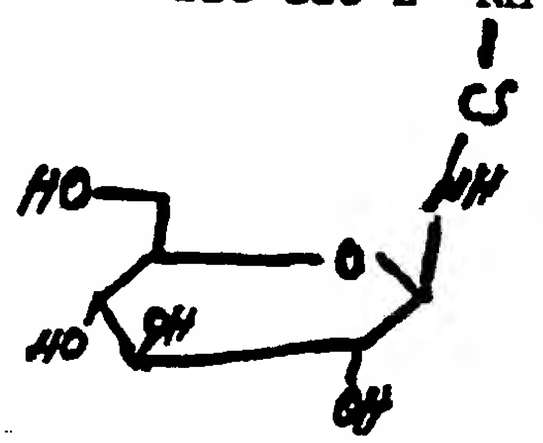
(7)

Example 6

Attachment of glucose to a 2'-amino-modified oligonucleotide

A solution of the oligonucleotide (6) (100 OD_{260} units) in 0.2M sodium phosphate (pH 7.0; 70µl) is added to an Eppendorf tube containing a solution of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (1 mg) in dimethylformamide (30 µl) and the resulting mixture is stirred for 15 sec. and then incubated at ambient temperature for 1 day. The mixture is purified by preparative reversed phase C_{18} HPLC to give the protected thiourea derivative which is then deprotected with aqueous ammonium hydroxide at ambient temperature and purified by reversed phase C_{18} HPLC to give the glucose oligonucleotide conjugate having the following structure:

AGT GTT CAG TTC CGU-2'-NH



It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

WHAT IS CLAIMED IS:

1. An oligonucleotide wherein at least one nucleotide unit is substituted at the 2' position with a moiety X, wherein X is $-(L)_n-R_1$, wherein L is a linker group, n is 0 or 1, and R_1 is selected from the group consisting of (a) amino acids; (b) peptides, polypeptides, and proteins; (c) dipeptide mimics; (d) sugars; (e) sugar phosphates; (f) neurotransmitters; (g) hormones; (h) poly (hydroxypropylmethacrylamide); (i) polyethyleneimine; (j) dextrans; (k) polymaleic anhydride; (l) cyclodextrins; (m) starches; (n) steroids; (o) acridine; and (p) vitamins, with the proviso that the vitamin is not biotin.

2. The oligonucleotide of Claim 1 wherein the at least one nucleotide unit is the 3' terminal nucleotide unit.

3. The oligonucleotide of Claim 1 wherein the phosphorus containing moiety of the at least one nucleotide unit is a modified phosphate moiety.

4. The oligonucleotide of Claim 1 wherein R_1 is a peptide, polypeptide or protein.

5. The oligonucleotide of Claim 4 wherein R_1 is polylysine.

6. The oligonucleotide of Claim 1 wherein R_1 is an amino acid.

7. The oligonucleotide of Claim 1 wherein R_1 is a dipeptide mimic.

8. The oligonucleotide of Claim 1 wherein R_1 is a sugar.

9. The oligonucleotide of Claim 1 wherein R_1 is a sugar phosphate.

10. The oligonucleotide of Claim 1 wherein R_1 is a neurotransmitter.

11. The oligonucleotide of Claim 1 wherein R_1 is a hormone.

12. The oligonucleotide of Claim 1 wherein R_1 is polyethyleneimine.

13. The oligonucleotide of Claim 1 wherein R_1 is poly (hydroxypropylmethacrylamide).

14. The oligonucleotide of Claim 1 wherein R_1 is a dextran.

15. The oligonucleotide of Claim 1 wherein R_1 is polymaleic anhydride.

16. The oligonucleotide of Claim 1 wherein R_1 is a cyclodextrin.

17. The oligonucleotide of Claim 1 wherein R_1 is a starch.

18. The oligonucleotide of Claim 1 wherein R_1 is a steroid.

19. The oligonucleotide of Claim 1 wherein R_1 is acridine.

20. The oligonucleotide of Claim 1 wherein R_1 is a vitamin selected from the group consisting of Vitamin A, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Vitamin C, Vitamin D, Vitamin E, folic acid, and nicotinic acid.

21. The oligonucleotide of Claim 1 wherein the oligonucleotide is a deoxyribonucleotide.

22. The oligonucleotide of Claim 1 wherein the oligonucleotide is a ribonucleotide.

23. The oligonucleotide of Claim 1 wherein the oligonucleotide includes ribonucleotide and deoxyribonucleotide moieties.

24. A composition for binding to an RNA, a DNA, a protein, or a peptide, comprising:

(a) an oligonucleotide wherein at least one nucleotide unit is substituted at the 2' position with a moiety X,

wherein X is $-(L)_n-R_1$, wherein L is a linker group, n is 0 or 1, and R_1 is selected from the group consisting of (i) amino acids; (ii) peptides, polypeptides, and proteins; (iii) dipeptide mimics; (iv) sugars, (v) sugar phosphates; (vi) neurotransmitters; (vii) growth factors; (viii) poly (hydroxy-propylmethacrylamide); (ix) polyethyleneimine; (x) dextrans; (xi) polymaleic anhydride; (xii) cyclodextrins; (xiii) starches; (xiv) steroids; (xv) acridine; and (xvi) vitamins, with the proviso that the vitamin is not biotin; and

(b) an acceptable pharmaceutical carrier, wherein said oligonucleotide is present in an effective binding amount to an RNA, a DNA, a protein, or a peptide.

25. The composition of Claim 24 wherein the at least one nucleotide unit is the 3' terminal nucleotide unit.

26. The composition of Claim 24 wherein the phosphorus containing moiety of the at least one nucleotide unit is a modified phosphate moiety.

27. The composition of Claim 24 wherein R_1 is a peptide, polypeptide, or protein.

28. The composition of Claim 27 wherein R_1 is polylysine.

29. The composition of Claim 24 wherein R_1 is an amino acid.

30. The composition of Claim 24 wherein R_1 is a dipeptide mimic.

31. The composition of Claim 24 wherein R_1 is a sugar.

32. The composition of Claim 24 wherein R_1 is a sugar phosphate.

33. The composition of Claim 24 wherein R_1 is a neurotransmitter.

34. The composition of Claim 24 wherein R_1 is a hormone.

35. The composition of Claim 24 wherein R_1 is polyethyleneimine.

36. The composition of Claim 24 wherein R_1 is poly (hydroxypropylmethacrylamide).

37. The composition of Claim 24 wherein R_1 is a dextran.

38. The composition of Claim 24 wherein R_1 is polymaleic anhydride.

39. The composition of Claim 24 wherein R_1 is a cyclodextrin.

40. The composition of Claim 24 wherein R_1 is a starch.

41. The composition of Claim 24 wherein R_1 is a steroid.

42. The composition of Claim 24 wherein R_1 is acridine.

43. The composition of Claim 24 wherein R_1 is a vitamin selected from the group consisting of Vitamin A, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Vitamin C, Vitamin D, Vitamin E, folic acid, and nicotinic acid.

44. The composition of Claim 24 wherein the oligonucleotide is a deoxyribonucleotide.

45. The composition of Claim 24 wherein the oligonucleotide is a ribonucleotide.

46. The composition of Claim 24 wherein the oligonucleotide includes ribonucleotide and deoxyribonucleotide moieties.

47. In a process wherein an oligonucleotide is administered for binding to an RNA, a DNA, a protein, or a peptide, the improvement comprising:

administering to a host an effective binding amount of an oligonucleotide wherein at least one nucleotide unit is substituted at the 2' position with a moiety X, wherein X is

$-(L)_n-R_1$, wherein L is a linker group, n is 0 or 1, and R_1 is selected from the group consisting of (a) amino acids; (b) peptides, polypeptides, and proteins; (c) dipeptide mimics; (d) sugars; (e) sugar phosphates; (f) neurotransmitters; (g) growth factors; (h) poly (hydroxy-propylmethacrylamide); (i) polyethyleneimine; (j) dextrans; (k) polymaleic anhydride; (l) cyclodextrins; (m) starches; (n) steroids; (o) acridine; and (p) vitamins, with the proviso that the vitamin is not biotin.

48. The process of Claim 47 wherein the at least one nucleotide unit is the 3' terminal nucleotide unit.

49. The process of Claim 47 wherein the phosphorus containing moiety of the at least one nucleotide unit is a modified phosphate moiety.

50. The process of Claim 47 wherein R_1 is a peptide, polypeptide, or protein.

51. The process of Claim 50 wherein R_1 is polylysine.

52. The process of Claim 47 wherein R_1 is an amino acid.

53. The process of Claim 47 wherein R_1 is a dipeptide mimic.

54. The process of Claim 47 wherein R_1 is a sugar.

55. The process of Claim 47 wherein R_1 is a sugar phosphate.

56. The process of Claim 47 wherein R_1 is a neurotransmitter.

57. The process of Claim 47 wherein R_1 is a hormone.

58. The process of Claim 47 wherein R_1 is polyethylene imine.

59. The process of Claim 47 wherein R_1 is poly (hydroxypropylmethacrylamide).

60. The process of Claim 47 wherein R_1 is a dextran.

61. The process of Claim 47 wherein R_1 is polymaleic anhydride.

62. The process of Claim 47 wherein R_1 is a cyclodextrin.

63. The process of Claim 47 wherein R_1 is a starch.

64. The process of Claim 47 wherein R_1 is a steroid.

65. The process of Claim 47 wherein R_1 is acridine.

66. The process of Claim 47 wherein R_1 is a vitamin selected from the group consisting of Vitamin A, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Vitamin C, Vitamin D, Vitamin E, folic acid, and nicotinic acid.

67. The process of Claim 47 wherein the oligonucleotide is a deoxyribonucleotide.

68. The process of Claim 47 wherein the oligonucleotide is a ribonucleotide.

69. The process of Claim 47 wherein the oligonucleotide includes ribonucleotide and deoxyribonucleotide moieties.

70. A probe for determining the presence of a target DNA or RNA sequence, comprising:

an oligonucleotide wherein at least one nucleotide unit is substituted at the 2' position with a moiety X, wherein X is $-(L)_n-R_1$, wherein L is a linker group, n is 0 or 1, and R_1 is selected from the group consisting of (a) amino acids; (b) peptides, polypeptides, and proteins; (c) dipeptide mimics; (d) sugars; (e) sugar phosphates; (f) neurotransmitters; (g) hormones; (h) poly (hydroxypropylmethacrylamide); (i) polyethyleneimine; (j) dextrans; (k) polymaleic anhydride; (l) cyclodextrins; (m) starches; (n) steroids; (o) acridine; and (p) vitamins, with the proviso that the vitamin is not biotin.

71. The oligonucleotide of Claim 18 wherein the steroid is a sterol.

72. The composition of Claim 41 wherein the steroid is a sterol.

73. The process of Claim 64 wherein the steroid is a sterol.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04144

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; A61K 31/70; C07H 21/02
US CL :435/6; 514/44; 536/24.3 & 24.5

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 514/44; 536/24.3 & 24.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

The hybrid DNA/RNA sequence disclosed at p. 31 of the specification was subjected to a "sequence search" of relevant nucleic acid databases.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A, 91/08312, (HENDRICKS ET AL.) 13 June 1991, entire document.	1-73
A	WO, A, 92/16636, (BOURSNELL ET AL.), 01 October 1992, entire document.	1-73
A	EP, A, 0,402,132, (SHIMADA ET AL.), 12 December 1990, entire document.	1-73
A	WO, A, 91/10671, (COOK ET AL.), 25 July 1991, entire document.	1-73
A	US, A, 4,904,582, (TULLIS), 27 February 1990, entire document.	1-73

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
24 JUNE 1993

Date of mailing of the international search report
01 JUL 1993

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/04144

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A, 91/06556, (BUHR ET AL.), 16 May 1991, entire document.	1-73
A	CHEMICAL REVIEWS, vol. 90(4), issued June 1990, E. Uhlmann et al., "Antisense Oligonucleotides: A New Therapeutic principle," pp. 543-584, particularly p. 558.	1-73